SUPPLEMENTARY INFORMATION

Supplementary information S4 (Box). Epigenetic mechanisms contribute to incubation of cocaine craving

The first evidence of an involvement of epigenetic mechanisms came from a rat study of gene expression following a cocaine self-administration regimen leading to incubation of cocaine craving. Decreased mRNA levels for four immediate early genes including EGR1 were observed in the NAc and mPFC during the first 100 days of abstinence; in the mPFC, histone H3 acetylation at the promotor for EGR1 was decreased after 1 and 10 days of abstinence (longer abstinence periods were not evaluated)\(^1\). A follow up study using whole-genome expression analysis found that 76% of all gene expression changes in the NAc and 33% in mPFC developed during abstinence (i.e., they were not detected on WD1 but were present on WD10 and/or WD100), consistent with a role in the incubation of craving\(^2\). A proteomics study of mPFC tissue from identically treated rats also found proteins that changed during abstinence\(^3\), but changes at the protein level did not correspond to those found with whole-genome expression analysis\(^2\).

A more recent study established a causal role for epigenetic mechanisms in the incubation of cocaine craving\(^4\). Compared to saline controls, the NAc of rats studied on WD1 showed altered methylation of many genes implicated in the action of cocaine. Some methylation changes found on WD1 persisted on WD30, some increased or decreased between WD1 and WD30, and others were evident only on WD30. DNA methylation patterns were further altered by cue-induced seeking tests - these tests generally reversed the methylation changes that occurred during abstinence, and this reversal was much more robust when the test was conducted on WD30, when craving had incubated, than on WD1. Intra-NAc infusion of a DNA methyltransferase inhibitor (RG108) on the two days preceding a seeking test abolished expression of incubation on WD30, whereas infusion of a methyl donor enhanced cocaine seeking. Both effects persisted for 1 month, suggesting that DNA methylation is required to sustain incubation. Interestingly, RG108 decreased GluA1 protein\(^5\), suggesting that inhibition of DNA methylation may eliminate incubation by interfering with formation of the homomeric GluA1 receptors that sustain incubation after prolonged withdrawal\(^6\). Finally, the authors selected two proteins whose genes were demethylated by RG108 (estrogen receptor-1 and cyclin-dependent kinase 5). Pharmacological targeting of these proteins decreased cocaine seeking on WD30, suggesting that their epigenetic regulation is important for incubation, although some questions of behavioural selectivity exist given that drug seeking was decreased below WD1 levels\(^4\). The dorsal striatum also exhibits changes in epigenetic proteins in concert with incubation of methamphetamine seeking\(^6\).

References